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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/784,985				3790

7590 05/07/2003

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EXAMINER
NOGUEROLA, ALEXANDER STEPHAN

ART UNIT	PAPER NUMBER
1753	

DATE MAILED: 05/07/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/784,985	Applicant(s) BAYLELY ET AL.	
	Examiner ALEX NOGUEROLA	Art Unit 1753	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-45* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☒ Claim(s) *5-11 and 15-20* is/are allowed.

6)☒ Claim(s) *1-4,13,14,21-25,27-29 and 32-54* is/are rejected.

7)☒ Claim(s) *12,26,30 and 31* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *15 February 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *3* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

Double Patenting

1. Claim 22 is objected to under 37 CFR 1.75 as being a substantial duplicate of claim 24. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k).

Claim Rejections - 35 USC § 112

2. Claims 13 and 14 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention:

a) Claim 13 has a list of possible amino acids that does not include any of the amino acids listed in claim 12, from which it depends. It is not clear how claim 12 further limits claim 13.

Note that dependent claims will have the deficiencies of base and intervening claims.

Claim Rejections - 35 USC § 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

4. The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

5. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

6. Claims 1-4, 21-25, 27, 28, and 35-38 are rejected under 35 U.S.C. 102(b) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Walker et al. ("A pore-forming protein with a metal-actuated switch," *Protein Engineering*, vol. 7, no. 5, pp. 655-662, 1994) ("Walker I") in view of Walker et al. ("Key Residues for Membrane Binding, Oligomerization, and Pore Forming activity of staphylococcal α-Hemolysin Identified by Cysteine Scanning Mutagenesis and Targeted Chemical Modification," *The Journal of Biological Chemistry*, vol. 270, No. 39, Issue of September 29, pp. 23065-23071, 1995) ("Walker II") and Tomich et al. (US 5,368,712).

Addressing Claims 1 and 21, Walker I teaches a mutant staphylococcal α-Hemolysin polypeptide comprising a heterologous amino acid, wherein the heterologous amino acid binds an analyte and wherein the polypeptide assembles into a pore assembly. See the abstract; *Table I* on page 659; and the second full paragraph in the second column on page 660. Walker I discloses that the polypeptide assembles into a heterohexameric pore assembly, not a heptameric pore assembly as claimed. There appears to be some confusion, though, in the art as to whether staphylococcal α-Hemolysin is a hexamer or heptamer. Applicant's specification, on page 11, states, "α-HL is a 293 amino acid polypeptide secreted by *Staphylococcus aureus* as a water-soluble monomer that assembles into lipid bilayers to form a heptameric pore." Walker II also states that α-HL is a 293 amino acid polypeptide secreted by *Staphylococcus aureus* as a water-soluble monomer that assembles to form a heptameric pore. See the abstract. However, Walker I states "Staphylococcal α-hemolysin, a pore-forming exotoxin, is a polypeptide of 293 amino acids that is secreted by *Staphylococcus aureus* as a water soluble monomer. It assembles to form hexameric pores in lipid bilayers." So, Applicant's staphylococcal α-Hemolysin and

Walker I's staphylococcal α-Hemolysin may actually both be heptamers or both may be hexamers.

The following applies if Applicant's staphylococcal α-Hemolysin is actually a heptamer and Walker I's staphylococcal α-Hemolysin is actually a hexamer. Walker II teaches performing scanning mutagenesis and targeted chemical modification of staphylococcal α-Hemolysin to determine residues and regions of the polypeptide important for binding, heptamer formation, and pore activity. See the abstract; the first full paragraph in the first column on page 23066; and Figure 5. One with ordinary skill in the art would recognize that the technique of Walker II could also be applied to hexameric staphylococcal α-Hemolysin, a homolog of heptameric staphylococcal α-Hemolysin, to determine regions of the polypeptide important for binding, heptamer formation, and pore activity. Tomich et al. teaches selecting a native polypeptide pore and modifying the polypeptide pore sequence to optimize measurements on an analyte of interest (the abstract and col. 4, ln. 56-63).

It would have been obvious to one with ordinary skill in the art at the time the invention was made to mutate in the manner of Walker I a staphylococcal α-Hemolysin that forms a heptameric pore, such as the one mapped by Walker II, instead of a staphylococcal α-Hemolysin that forms a hexameric pore, because then as taught by Tomich et al. the pore can be optimized for the analyte of interest. Also, since hexameric staphylococcal α-Hemolysin is just a homolog of heptameric staphylococcal α-Hemolysin, the homologs differing from each other by one-repeating unit, it is an obvious variant. .

Barring evidence to the contrary, such as unexpected results, the choice to mutate a staphylococcal α-Hemolysin that forms a heptameric pore, such as the one mapped by Walker II, instead of a staphylococcal α-Hemolysin that forms a hexameric pore is just a matter of optimizing properties of the pore. Indeed, since hexameric staphylococcal α-Hemolysin is just a homolog of heptameric staphylococcal α-Hemolysin, the homologs differing from each other by one-repeating unit, one with ordinary skill in the art would expect the differences in properties to be slight.

Addressing Claim 2, as seen from the abstract of Walker II the pore spans a membrane.

Addressing Claim 3, the last paragraph in the first column on page 661 of Walker I, bridging to the second column, states that the heterologous amino acid is within the lumen of the transmembrane channel.

Addressing Claim 4, the heterologous amino acids replaces residues 130-134, which is within the stem domain according to Applicant's definition on page 2 of the specification.

Addressing Claims 22 and 24, the pore assembly of Walker I as modified by Walker II has the formula WT_6MUT_1.

Addressing Claim 23, as seen from the abstract of Walker II the pore spans a membrane. Also, as seen from the abstract of Walker I the heterologous amino acid binds Zn^{+2}.

Addressing Claims 25, 27, and 28, Walker I as modified by Walker II disclose H5 and αHL (first column on page 656 and *Table I* on page 659 of Walker I, for example, and Figure 2 of Walker II). Again, barring evidence to the contrary, such as unexpected results, using a homolog of the polypeptide used by Walker I as modified by Walker II is just a matter of optimizing the desired properties. Walker II teaches how to systematically determine the effects of replacement amino acids on the properties of the polypeptide pore assembly. For claim 28 note that the pore assembly of Walker I as modified by Walker II has the formula WT_6MUT_1.

Addressing Claims 35-38, the base-line in Figure 5 shows current through the pore when the pore is contact with solution that does not contain the analyte, Zn^{+2}. Whether to use one pore or more than one pore is based on whether only the characteristics of the pore are be determined, and if actual measurements are to be made on a sample, the concentration of analyte in the sample and the sensitivity of the detection means.

7. Claim 39 is rejected under 35 U.S.C. 103(a) as being unpatentable over Walker et al. ("A pore-forming protein with a metal-actuated switch," *Protein Engineering*, vol. 7, no. 5, pp. 655-662, 1994) ("Walker I") in view of Walker et al. ("Key Residues for Membrane Binding, Oligomerization, and Pore Forming activity of staphylococcal α-Hemolysin Identified by Cysteine Scanning Mutagenesis and Targeted Chemical Modification," *The Journal of Biological Chemistry*, vol. 270, No. 39, Issue of September 29, pp. 23065-23071, 1995) ("Walker II") and Tomich et al. (US 5,368,712) as applied to claims 1-3, 21-25, 27, 28, and 35-

38 above, and further in view of Kasianowicz et al. ("Genetically Engineered Pores as Metal Ion Biosensors," *Mat.Res. Soc. Symp. Proc.*, Vol. 330 (1994), 330(Biomolecular Materials by Design), 217-23). Although Walker I as modified by Walker II only appear to disclose Zn^{+2} as an analyte, Kasianowicz et al. disclose that Co(II), Ni(II), and Cd(II) can also be analytes (the abstract and Figure 1).

8. Claims 29 and 32-34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Walker et al. ("A pore-forming protein with a metal-actuated switch," *Protein Engineering*, vol. 7, no. 5, pp. 655-662, 1994) ("Walker I") in view of Walker et al. ("Key Residues for Membrane Binding, Oligomerization, and Pore Forming activity of staphylococcal α-Hemolysin Identified by Cysteine Scanning Mutagenesis and Targeted Chemical Modification," *The Journal of Biological Chemistry*, vol. 270, No. 39, Issue of September 29, pp. 23065-23071, 1995) ("Walker II") and Tomich et al. (US 5,368,712) as applied to claims 1-3, 21-25, 27, and 28 above, and further in view of Kasianowicz ("Detecting and characterizing analytes with an ion channel," Book of Abstracts, 213th ACS National Meeting, San Francisco, April 13-17). Although Walker I as modified by Walker II do not specifically state providing their pore assembly in digital biosensor, they appear to have contemplated such a modification because they state "genetically-engineered pore-forming proteins might make useful components of metal ion sensors" (the abstract). In any event, using synthetic or mutated polypeptides as a pore assembly in a digital biosensor was known at the time of the invention as shown by Tomich et al.

(the abstract and Figure 1) and also by Kasianowicz. It would have been obvious to one with ordinary skill in the art at the time the invention was made to use the pore assembly of claim 21 in a biosensor as taught by Tomich et al. or Kasianowicz because then the biosensor will be able to selectively monitor Zn^{+2}. In other words, the choice of pore assembly is determined by the analyte of interest. Indeed, Kasianowicz footnotes Walker I.

9. Claims 33 and 34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Walker et al. ("A pore-forming protein with a metal-actuated switch," *Protein Engineering*, vol. 7, no. 5, pp. 655-662, 1994) ("Walker I") in view of Walker et al. ("Key Residues for Membrane Binding, Oligomerization, and Pore Forming activity of staphylococcal α-Hemolysin Identified by Cysteine Scanning Mutagenesis and Targeted Chemical Modification," *The Journal of Biological Chemistry*, vol. 270, No. 39, Issue of September 29, pp. 23065-23071, 1995) ("Walker II") and Tomich et al. (US 5,368,712) as applied to claims 1-3, 21-25, 27, and 28 above, and further in view of Kasianowicz ("Detecting and characterizing analytes with an ion channel," Book of Abstracts, 213th ACS National Meeting, San Francisco, April 13-17) as applied to claims above, and further in view of Kasianowicz et al. ("Genetically Engineered Pores as Metal Ion Biosensors," *Mat.Res. Soc. Symp. Proc.*, Vol. 330 (1994), 330(Biomolecular Materials by Design), 217-23).

Both single channel and multi-channel devices are disclosed by Walker I as modified by Walker II and Tomich et al. Additionally, Kasianowicz et al. teach some reasons for using a single channel device or a multi-channel device on page 221.

10. Claims 40-44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Walker et al. ("A pore-forming protein with a metal-actuated switch," *Protein Engineering*, vol. 7, no. 5, pp. 655-662, 1994) ("Walker I") in view of Walker et al. ("Key Residues for Membrane Binding, Oligomerization, and Pore Forming activity of staphylococcal α-Hemolysin Identified by Cysteine Scanning Mutagenesis and Targeted Chemical Modification," *The Journal of Biological Chemistry*, vol. 270, No. 39, Issue of September 29, pp. 23065-23071, 1995) ("Walker II") and Tomich et al. (US 5,368,712) as applied to claims 1-3, 21-25, 27, 28, and 35-38 above, and further in view of Kasianowicz et al. ("Genetically Engineered Pores as Metal Ion Biosensors," *Mat.Res. Soc. Symp. Proc.*, Vol. 330 (1994), 330(Biomolecular Materials by Design), 217-23).

Walker I as modified by Walker II and Tomich et al. teach detecting an electrical current through one or more pores to identify an analyte, Zn^{+2}, (Figure 5 of Walker I and col. 4, ll. 4-63 in Tomich et al.). Whether to use one pore or more than one pore is based on whether only the characteristics of the pore are be determined, and, if actual measurements are to be made on a sample, the concentration of analyte in the sample and the sensitivity of the detection means. Kasianowicz et al. teach some additional reasons for using a single channel device or a multi-channel device on page 221.

Although Walker I as modified by Walker II and Tomich et al. teach detecting an analyte, Zn^{+2}, based on the current signal, they do not mention detecting the analyte, $Zn^{+2,}$ in a mixture of analytes and using the concurrence of the measured signal with a standard current signature of a known analyte to indicate the identity of the unknown analyte. As for detecting the analytes in a mixture of analytes, it would have been obvious to one with ordinary skill in the art at the time

the invention was made to do so because as shown by Kasianowicz et al. the pore assembly of Walker I as modified by Walker II and Tomich et al. is selective for certain analytes for example, as seen in Figure 1 of Kasianowicz et al. Mg and Ca have no effect on ion current through the pore assembly). As for using a standard current signature to indicate the identity of unknown analyte, it would have been obvious to one with ordinary skill in the art at the time the invention was made to do so because as taught by Kasianowicz et al. analytes to which the pore assembly is selective have distinguishable current profiles, so further selectivity can be obtained by "analyzing the ion channel current noise" (first full paragraph on page 220 and col. 12, ll. 1-9 of Tomich et al.).

Addressing Claim 45, although Walker I as modified by Walker II only appear to disclose Zn^{+2} as an analyte, Kasianowicz et al. disclose that Co(II), Ni(II), and Cd(II) can also be analytes (the abstract and Figure 1).

Allowable Subject Matter

11. Claims 15-20 are allowed.

12. Claims 5-12, 26, and 30 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

13. The following is a statement of reasons for the indication of allowable subject matter:

a) Claims 5: Walker I (*Protein Engineering*, vol. 7, no. 5, pp. 655-662, 1994) as modified by Walker II only discloses consecutive heterologous amino aids in the stem domain;

b) Claims 6-11 depend directly or indirectly from allowable claim 5;

c) Claims 12, 26, and 31: Walker I (*Protein Engineering*, vol. 7, no. 5, pp. 655-662, 1994) as modified by Walker II (*The Journal of Biological Chemistry*, vol. 270, No. 39, Issue of September 29, pp. 23065-23071, 1995) only discloses using histidine or cysteine as replacement amino acids;

d) Claims 15 and 30: Walker I only discloses consecutive heterologous amino acids in a stem domain; and

e) Claims 16-20 depend directly or indirectly from allowable claim 15.

14. Any inquiry concerning this communication or earlier communications from the examiner should be directed to ALEX NOGUEROLA whose telephone number is (703) 305-5686. The examiner can normally be reached on M-F 8:30 - 5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, NAM NGUYEN can be reached on (703) 308-3322. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 872-9310 for regular communications and (703) 872-9311 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0661.

Alex Noguerola
April 23, 2003